U.S.D.A. FOREST SERVICE
RESEARCH NOTE RM-143

FOREST SERVICE
U.S. DEPARTMENT OF AGRICULTURE

ROCKY MOUNTAIN FOREST AND RANGE EXPERIMENT STATION

Assimilation Chamber for Measuring Carbon Dioxide Exchange of Tree Seedlings in the Laboratory

Frank Ronco[1]

An apparatus to measure photosynthesis and respiration is described in sufficient detail to facilitate construction. Design features include portability, rapidity of measurement, and long-term reliability of the temperature control system. Since the temperature controls and other components are separate from the chamber, plants of different sizes can be measured simply by interchanging chambers of different dimensions.

An open system with a plexiglass assimilation chamber for measuring gaseous exchange of carbon dioxide (CO_2) in potted Engelmann spruce (Picea engelmannii Parry) and lodgepole pine (Pinus contorta Dougl.) seedlings under field conditions has been described.[2] Performance was satisfactory in the field, but not in the laboratory where experimental conditions required a different system and more elaborate equipment.

The water jacket in the plexiglass field chamber maintained acceptable temperatures under natural light, but could not filter and dissipate the heat from artificial lights with an intensity of 13,000 foot-candles—equivalent to natural light intensity during clear days at elevations where spruce grows. Secondly, reliable photosynthesis measurements are difficult to obtain in a laboratory with an open system, because CO_2 concentration can change from 300 to over 600 p.p.m. within a few minutes due to organic fuel combustion and CO_2 exhalation by persons in the laboratory area.

Since photosynthesis in an open system is determined by the reduction of CO_2 concentration of air after passing over a seedling, the difficulties encountered with a rapidly fluctuating CO_2 concentration are obvious. Consequently, a closed system—where gaseous exchange is measured by changes in CO_2 concentration over time—is more suitable for laboratory measurements. The apparatus described is a modification of units reported by Decker[3,4] and Brix.[5] Some of the desirable features of those units

[1] *Silviculturist, Rocky Mountain Forest and Range Experiment Station, with central headquarters maintained at Fort Collins, in cooperation with Colorado State University.*

[2] *Ronco, Frank. Assimilation chamber for outdoor measurements of photosynthesis of tree seedlings. U.S.D.A. Forest Serv. Res. Note RM-142, 4 pp., illus. 1969. Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colo.*

[3] *Decker, J. P. The effect of air supply on apparent photosynthesis. Plant Physiol. 22: 561-571. 1947.*

[4] *Decker, J. P. The effect of light intensity on photosynthetic rate in Scotch pine. Plant Physiol. 29: 305-306. 1954.*

[5] *Brix, H. The effect of water stress on the rates of photosynthesis and respiration in tomato plants and loblolly pine seedlings. Physiol. Plant. 15: 10-20. 1962.*

are incorporated, as well as innovations designed for versatility and operating convenience.

Advantages over similar devices are: compactness and portability; maintenance of a set temperature without overcompensation; reliability during continued operation; ease of plant insertion in the chamber; and interchangeability of chambers for different-sized plants.

Basically, the apparatus consists of an airtight plant chamber and systems for calibrating, cooling, drying and humidifying air, and measuring CO_2. Carbon dioxide concentrations are most conveniently and accurately determined with an infrared gas analyzer; the other systems are easily fabricated or assembled from standard laboratory equipment.

Function of Component Systems

The various systems are described in the following paragraphs; letters in parentheses identify component parts shown in figure 1.

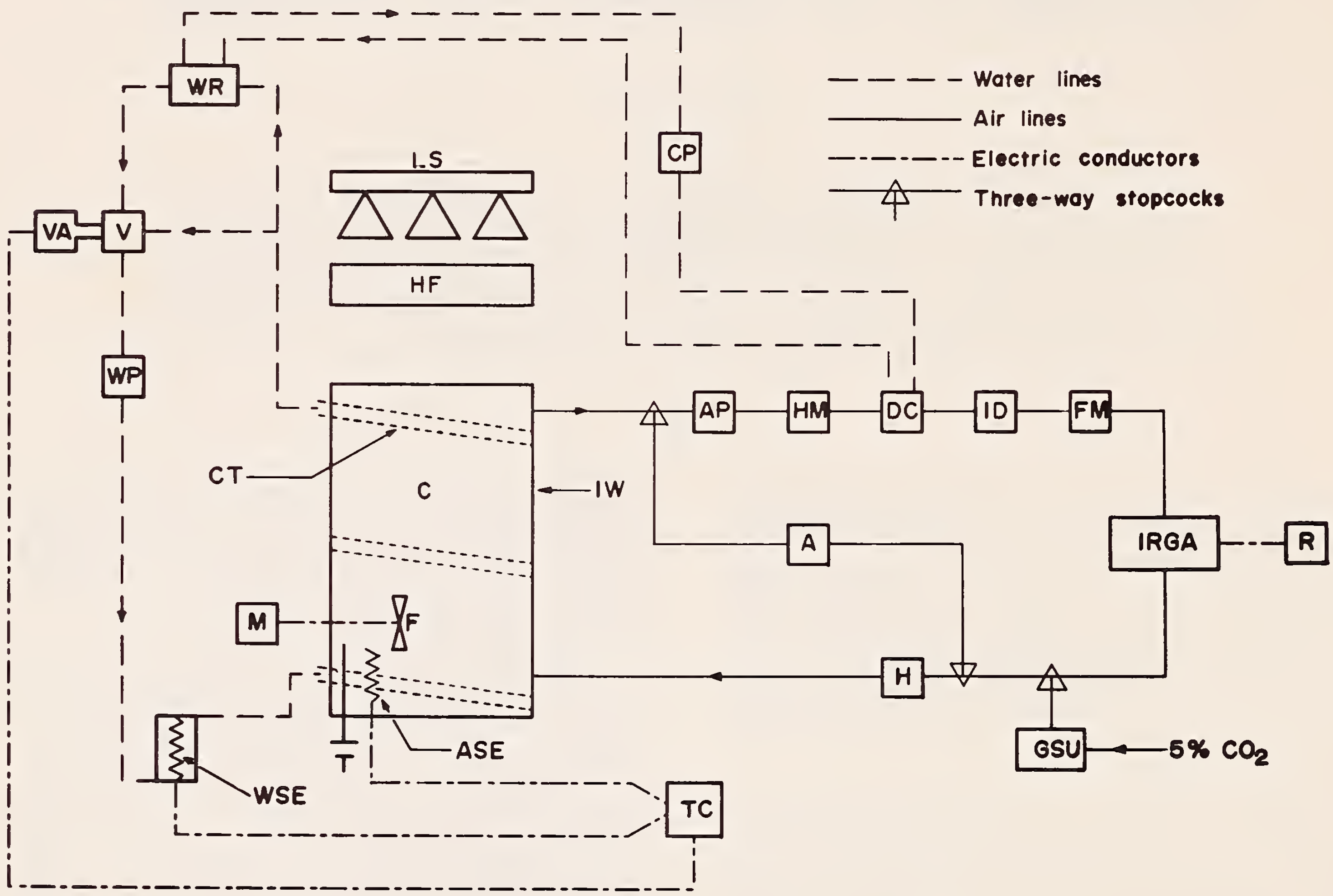

Figure 1.--Schematic diagram of closed-system apparatus for measuring photosynthesis.

A -	*ascarite column*	*IRGA* -	*infrared gas analyzer*
AP -	*air pump*	*IW* -	*internal copper wall of chamber*
ASE -	*air temperature sensing element*	*LS* -	*light source*
C -	*assimilation chamber*	*M* -	*fan motor*
CP -	*centrifugal water pump*	*R* -	*strip-chart recorder*
CT -	*copper tubing*	*T* -	*thermometer*
DC -	*water-cooled condenser*	*TC* -	*temperature controller*
F -	*fan*	*V* -	*3-way valve*
FM -	*flow meter*	*VA* -	*valve actuator*
GSU -	*gas sampling unit*	*HF* -	*heat filter*
H -	*humidifier*	*WP* -	*water pump*
HM -	*relative humidity meter*	*WR* -	*water reservoir and refrigerator*
ID -	*indicating drierite* ($CaSO_4$)	*WSE* -	*water temperature sensing element*

Air is drawn from a sealed chamber (C) by a Neptune Dyna-pump (AP),[6] and forced through a Serdex humidity meter (HM) to an air-drying system consisting of a water-cooled condenser (DC) and a cylinder filled with indicating drierite (ID). The drying condenser is cooled by recirculating chilled water with a centrifugal immersion pump (CP). Dried air moves through a flow meter (FM) into a Beckman 15A gas analyzer (IRGA) and returns to the chamber via a humidifier (H)—a gas-washing bottle. Carbon dioxide concentration and time are recorded on a Varian strip-chart recorder (R).

The light source (LS) is seven 300-watt spotlight bulbs, with a switch arrangement to illuminate bulbs in consecutive order. Most of the infrared radiation from the lights is removed by water in a heat filter (HF), and the portion not absorbed is dissipated by cold water flowing through copper tubing (CT) soldered to the inner wall (IW) of the chamber. An Eastern D-11 pump (WP) continuously recirculates the water in the copper tubing, which is maintained at a desired temperature by a regulated flow of chilled water from a refrigerated reservoir (WR). A fan (F) driven by a variable-speed stirrer motor (M), mounted outside the chamber, circulates air to increase cooling efficiency, and to prevent variable carbon dioxide concentrations. Air turbulence rates are variable from slow to rapid.

The temperature control system, manufactured by Johnson Service, is composed of five separate units: an air temperature sensing element (ASE), a water temperature sensing element (WSE), a temperature controller (TC), a proportional electro-hydraulic valve actuator (VA), and a 3-way valve (V). A low voltage signal—proportional to the temperature sensed by the air and water sensing elements—is transmitted by the electronic controller to the valve actuator mechanism, which positions the 3-way valve in proportion to the temperature sensed at the elements. When the controls are properly calibrated, the actuator will position the valve between its extreme limits of travel so that the valve-intake ports leading to the water reservoir and the copper tubing are partially open. As the valve slowly modulates in response to temperature deviations from the temperature set on the controller, a gradual change in the flow of chilled water from the reservoir will be accompanied by an equally gradual but inverse change in the flow of water already in the system. These gradual changes in flow rates prevent overcompensation in the heating-cooling cycle typical of relay-circuit controls, and make it possible to maintain chamber temperatures within 0.1°C. Chamber temperatures of 10° to 40°C. are possible under a full light load. Temperatures are recorded with a bimetallic dial thermometer (T), but the system should be calibrated by a sensitive thermocouple thermometer.

Valve movement is largely controlled by the air temperature sensing element, but somewhat finer control is obtained with the water sensing element. Since there is a time lag between chilled water entry into the copper tubing and its cooling effect inside the chamber, the latter element tends to reduce chilled water intake before such action is called for by the primary air sensor.

For photosynthesis determinations, the lights provide sufficient heat to raise chamber temperatures to commonly desired levels. Supplemental heat is required, however, to obtain temperatures above ambient when the chamber is darkened for respiratory determinations. That heat is obtained from an electric heating coil and relay activated by the temperature controller.

If duplicate photosynthesis measurements are desired, the CO_2 content of air in the chamber must be raised with a 5 percent CO_2 air mixture. That is accomplished by incorporating into the apparatus a gas sampling unit (GSU) composed of a gas sampling bulb with a 3-way stopcock at each end and a graduated 250-ml. separatory funnel used as a leveling bulb.

The calibrating system provides dry, CO_2-free air for the gas analyzer; two 3-way stopcocks bypass the chamber with a cylinder of Ascarite (A) that absorbs CO_2 from the air stream flowing through the analyzer.

Construction

This section presents general descriptions needed for understanding and constructing the apparatus, but fabrication techniques and assembly are left to craftsmen and researchers. Figures 2, 3, and 4 show the assembled apparatus, with the component parts lettered and identified in the figure captions.

The apparatus, except for the water reservoir, coolant pump, flow meter, and gas analyzer, is

[6] *Trade names and company names are used for the benefit of the reader and do not imply endorsement or preferential treatment by the U. S. Department of Agriculture.*

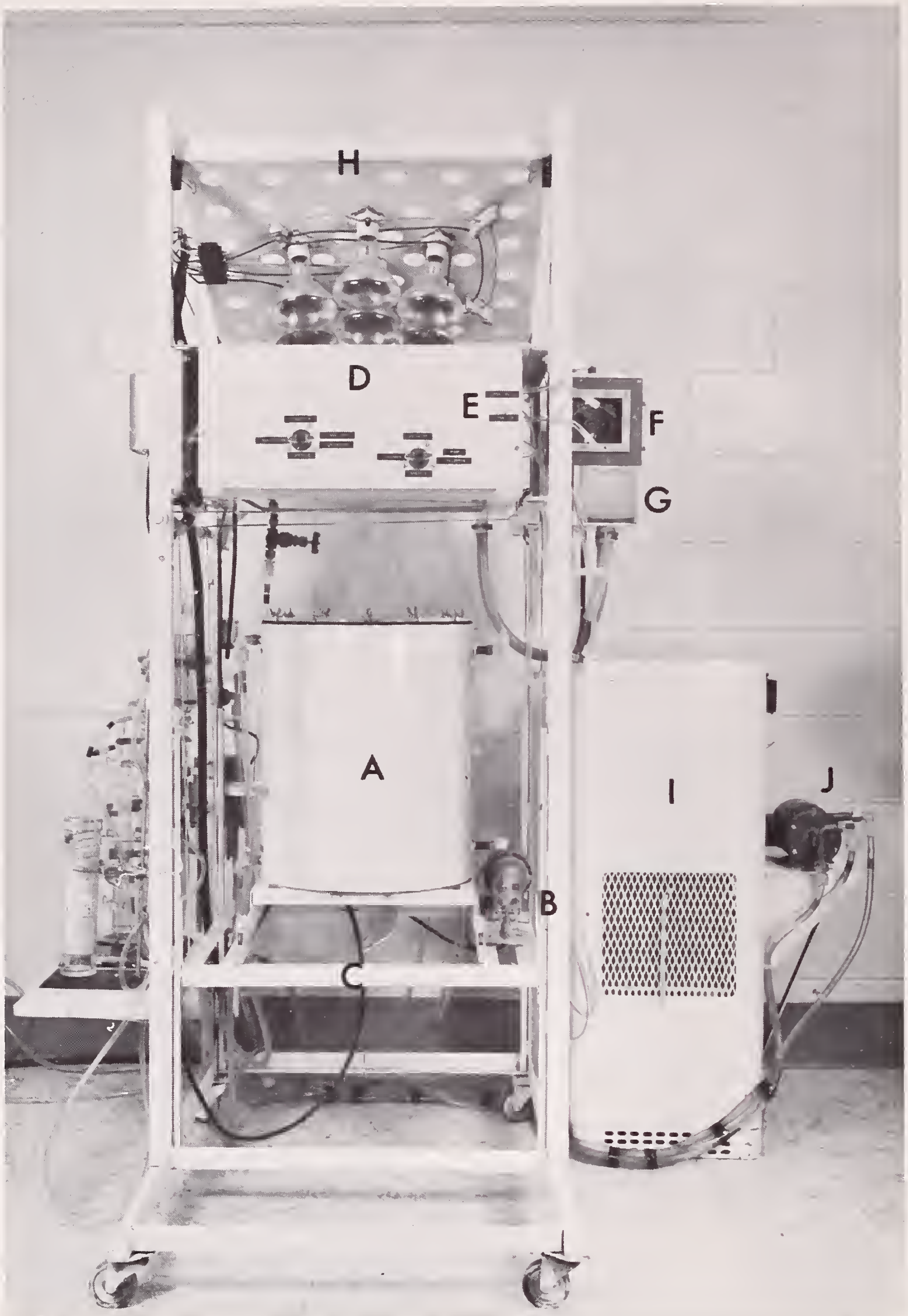

Figure 2.--Front view of closed-system apparatus for measuring carbon dioxide exchange of tree seedlings.

A - plant chamber
B - air pump
C - adjustable shelf
D - control panel with 3-way stopcocks of calibration system
E - inlet and outlet tubing to flow meter and gas analyzer
F - humidity meter
G - water-level control of heat filter
H - light bracket raised to show lamp arrangement
I - refrigeration unit and water reservoir
J - coolant circulating pump

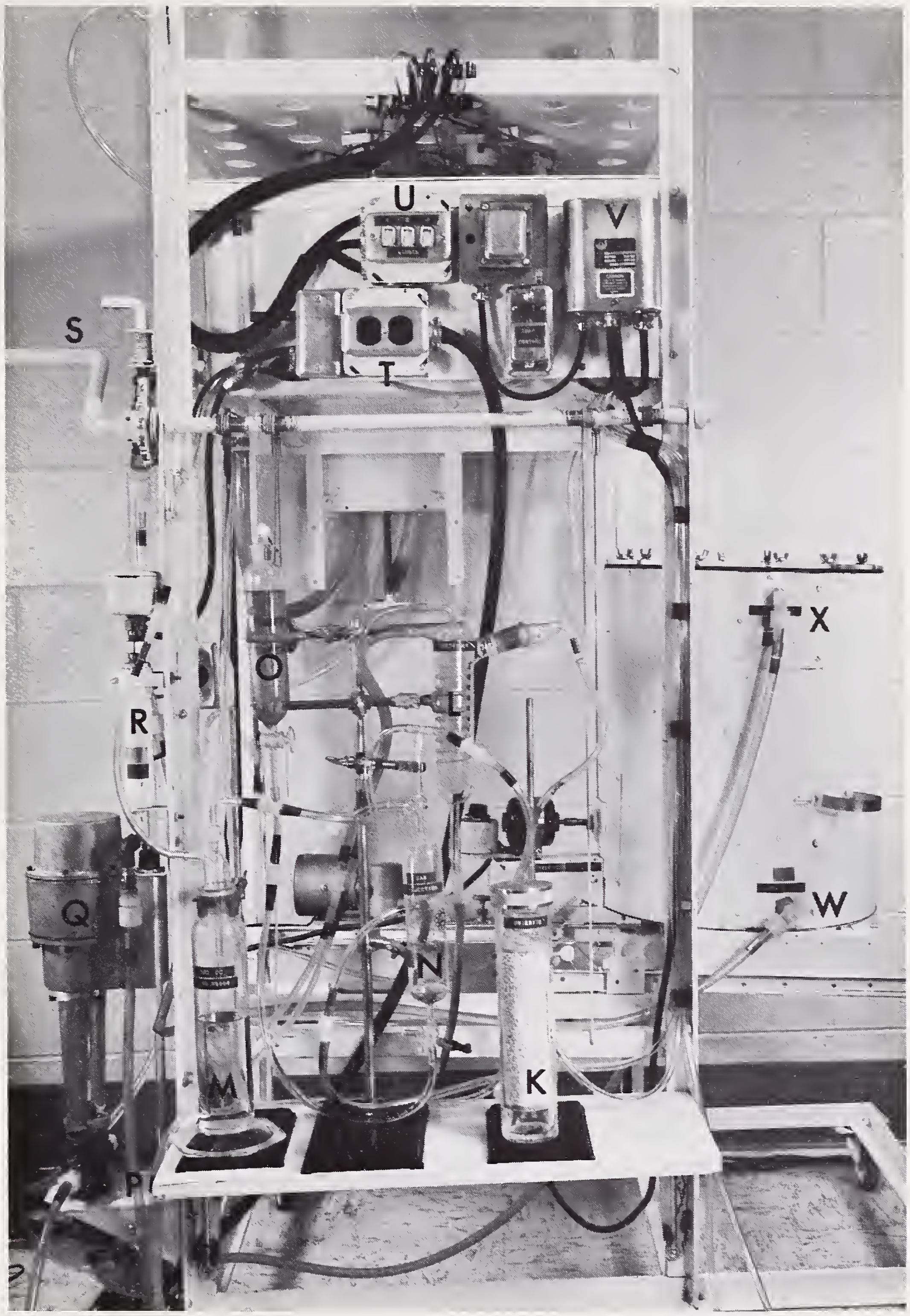

Figure 3.--Left side view of closed-system apparatus for measuring carbon dioxide exchange of tree seedlings.

K - drierite column
L - drying condenser
M - humidifier
N - bulb of gas sampling unit
O - separatory funnel of gas sampling unit
P - valve
Q - valve actuator
R - water temperature sensing element
S - lever and ratchet for vertical shelf adjustment
T - utility outlets
U - light switches
V - temperature controller
W - water inlet to chamber cooling coil
X - water outlet from chamber cooling coil

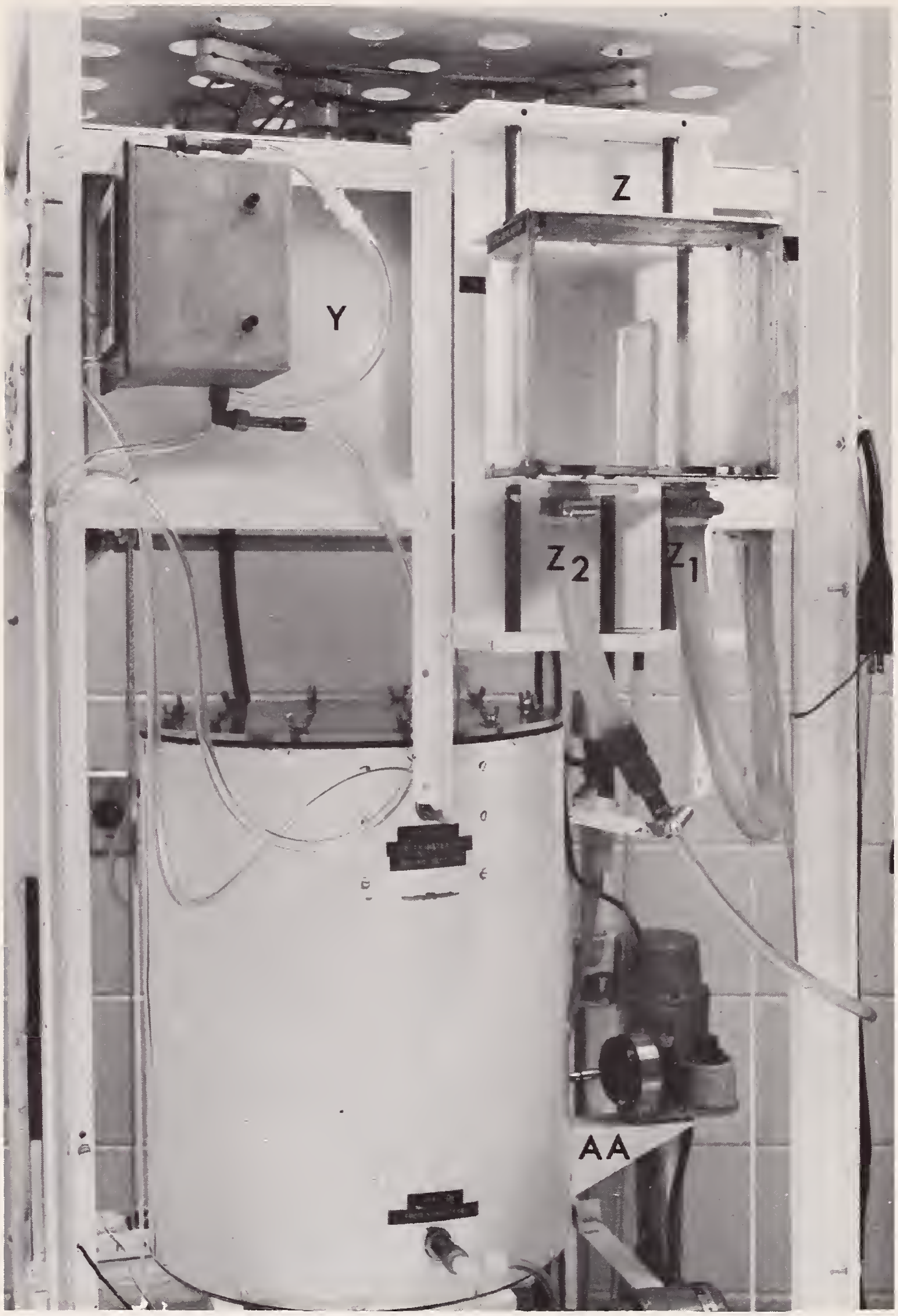

Figure 4.--Right side view of closed-system apparatus for measuring carbon dioxide exchange of tree seedlings.

Y - heat filter
Z - water-level control for heat filter
Z_1 - water inlet to water-level control
Z_2 - water outlet from water-level control
AA - fan motor and rheostat

mounted on a welded angle-iron framework with rubber-tired casters. An estimate of the overall dimensions can be obtained from the upright supports, which are 6 feet high and spaced 3 feet apart.

The chamber is a double-walled cylinder, 22 inches long with an inside diameter of 12 inches; the inner cylinder wall is copper and the outer galvanized sheet metal. The 2-inch air space between the walls—filled with insulating vermiculite—is maintained by two flat rings, resembling washers, machined from 0.500-inch-thick aluminum plates. Each ring is fitted with 12 equally spaced 0.250-inch bolts which are capped with wing-nuts to hold the sheet-metal bottom and 0.250-inch-thick plexiglass top of the chamber in position. Rubber O-rings form an airtight seal between the aluminum rings and end covers of the chamber. One-half inch copper tubing is coiled and soldered around the outside of the copper cylinder at 1-inch intervals.

The seedling pot must be placed in the chamber because of the one-piece bottom. Consequently, the pot must be sealed to prevent CO_2 respired by soil organisms from entering the chamber atmosphere. A circular 0.150-inch-thick plexiglass disk—cut to a diameter slightly smaller than the diameter of the pot and slotted to accommodate the seedling stem—is placed over the soil. Masking tape and modeling clay seal the cover in place.

An open aluminum tank, 10 inches deep with a plate glass bottom, is mounted below the light source for a heat filter. Tap water flows continuously into the tank to replace evaporative loss. An overflow outlet acts as a water-level control.

The water-level control unit is an enclosed plexiglass box, partially divided into two compartments by a vertical plastic wall—with a sharp diagonal crest—that extends from the floor halfway up the sides of the box. Water entering one compartment through vinyl tubing from the tank flows over the crest into the other compartment, then to a floor drain via additional tubing. By adjusting the height of the control unit with a threaded rod, the water level in the tank can be maintained at any desired level.

A water reservoir is made from a refrigerated drinking fountain in which the internal water piping is replaced with a 2-gallon rustproof cylindrical tank containing a coil of copper tubing connected to the refrigeration unit. A continuous-duty water pump is mounted on the side of the fountain to circulate coolant to the chamber. An immersion pump, the type used in evaporative household coolers with an output of 2 gallons per minute, is placed in the tank to circulate water to the air-drying condenser.

To facilitate changing seedlings, the chamber is mounted on an adjustable shelf which can be positioned vertically with a ratchet and cable device, and moved horizontally on sliding-drawer guides.

The sheet-metal bracket holding the spotlights can be pivoted to a vertical position to replace bulbs and clean the heat filter.

Connections between vinyl tubing—0.375-inch inside-diameter water lines and 0.250-inch inside-diameter air lines—and pipe fittings are made with polypropylene tube fittings. Polyethylene quick disconnects are used in appropriate locations to join sections of air lines for ease of maintenance and operation.

Possible air leakage into the chamber through the fan-shaft opening can be eliminated by a machined shaft-housing—fitted with two ball bearing cones and two grease seals—which is kept filled with light grease applied through a grease fitting in the housing.

The chamber as described may not be suitable for photosynthesis measurements where experimental objectives of sizes and kinds of plants are different. Since the chamber is separate from most of the equipment, however, chambers of different designs can be interchanged to meet various requirements while utilizing all other components of the apparatus.